Datasheet for ABIN5067614

# Hydroxyproline Assay Kit

3 Images | 2 Publications

Go to Product page

## Overview

| | |
|---|---|
| Quantity: | 96 tests |
| Application: | Biochemical Assay (BCA) |

## Product Details

| | |
|---|---|
| Purpose: | Hydroxyproline Assay Kit provides a convenient colorimetric method for the detection of total hydroxyproline from tissue, plasma, serum, or urine acid-hydrolysates. |
| Components: | 1. Hydroxyproline Standard : One 100 µL vial containing 1 mg/mL Hydroxyproline.<br>2. Assay Buffer : One 12 mL bottle.<br>3. Chloramine T Reagent : One 600 µL vial.<br>4. 2X Ehrlich's Concentrate : One 5 mL bottle.<br>5. Ehrlich's Diluent : One 5 mL bottle. 3 |

## Target Details

| | |
|---|---|
| Background: | Hydroxyproline is an amino acid that is synthesized from the irreversible post-translational hydroxylation of proline by prolyl hydroxylase. Hydroxyproline is found almost exclusively in the protein collagen, in the Y position of the repeating tripeptide Gly-X-Y. By allowing sharp twisting of the collagen helix, hydroxyproline helps to stabilize the structure of collagen. In addition to collagen, hydroxylation of proline has been observed on the transcription factor Hypoxia Inducible Factor (HIF- 1). Under normal oxygen conditions the protein EGLN1 hydroxylates HIF-1 alpha at proline 564, allowing ubiquitylation by the von Hippel-Lindau tumor suppressor (pVHL) and causing the targeting of HIF-1 for degradation by the proteasome. Since hydroxyproline has been found on so few proteins other than collagen, measurement of hydroxyproline has been used as a marker to quantify levels of collagen and/or gelatin (partial hydrolysis of collagen resulting in a mixture of protein and peptides). It is estimated that |

## Target Details

hydroxyproline makes up 13.5 % of collagen. In addition, hydroxyproline measurement has been used to identify certain diseases that involve breakdown of collagen. For example, increased levels of collagen have been measured in serum of Paget's Bone Disease. In addition, increased hydroxyproline levels have been correlated with prostatic carcinoma bone metastases, hepatic fibrosis, as well as melamine and cyanuric acid induced nephrotoxicity.

## Application Details

| | |
|---|---|
| Application Notes: | Optimal working dilution should be determined by the investigator. |
| Protocol: | First, the unknown samples or hydroxyproline standards are added to a 96 well plate. Then, a Chloramine T mixture is added to convert the hydroxyproline to a pyrrole. Finally, a 4-(Dimethylamino)benzaldehyde (DMAB) mixture (also known as Ehrlich's Reagent) is added to the well which reacts with the pyrrole to produce a chromophore and the absorbance of the plate is read at 540-560 nm. The content of hydroxyproline in the unknown samples is determined by comparison with a predetermined hydroxyproline standard curve. The provided reagents are sufficient for the evaluation of 96 assays including standards and unknown samples. |
| Reagent Preparation: | • Chloramine T Mixture: Incubate Chloramine T Reagent for 10-15 minutes at 37 °C. For each well to be measured, add 6 µL of Chloramine T Reagent to 94 µL of Assay Buffer. Mix well. Use this mixture within 3 hours of preparation and discard unused Chloramine T Mixture. Aliquot remainder of unused Chloramine T Reagent before returning to 4 °C storage to avoid multiple heat/cold cycles.<br>• Ehrlich's Reagent: For each well to be measured, mix 50 µL of 2X Ehrlich's Concentrate with 50 µL of Isopropanol/Perchloric Acid. Mix well. Use within 3 hours of preparation and discard unused Ehrlich's Reagent. Preparation of Standard Curve Prepare a dilution series of Hydroxyproline standards in the concentration range of 0 to 760 µM by diluting the Hydroxyproline Standard in distilled water (Table 1). 7.6 mM Hydroxyproline Distilled Water Hydroxyproline Standard Tubes Standard (µL) (µL) (µM) 1 10 90 760 2 50 of Tube #1 50 380 3 50 of Tube #2 50 190 4 50 of Tube #3 50 95 5 50 of Tube #4 50 47.5 6 50 0 Table 1. Preparation of Hydroxyproline Standards. 4 |
| Sample Preparation: | The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.<br>• Cells: Resuspend 3-6 x 106 cells in distilled water. Transfer 100 µL of cell suspension to a 0.5 mL or 2 mL Teflon capped, pressure tight vial and add 100 µL of 12 N hydrochloric acid. Hydrolyze the sample for 3 hours at 120 °C. Let cool briefly and then add 5 mg of activated charcoal*. Mix well by vortexing and then centrifuge at 10000 xg for 5 minutes. Recover the supernatant and transfer to a new tube. Store unused final sample at 4 °C.<br>• Tissue: Homogenize in 100 µL of distilled water for every 10 mg of tissue. Transfer 100 µL of |

## Application Details

| | |
|---|---|
| | tissue homogenate to a 0.5 mL or 2 mL Teflon capped, pressure tight vial and add 100 µL of 12 N hydrochloric acid. Hydrolyze the sample for 3 hours at 120 °C. Let cool briefly and then add 5 mg of activated charcoal*. Mix well by vortexing and then centrifuge at 10000 xg for 5 minutes. Recover the supernatant and transfer to a new tube. Store unused final sample at 4 °C.<br>• Urine, plasma, or serum: Transfer 100 µL of sample to a 0.5 mL or 2 mL Teflon capped, pressure tight vial and add 100 µL of 12 N hydrochloric acid. Hydrolyze the sample for 3 hours at 120 °C. Let cool briefly and then add 5 mg of activated charcoal*. Mix well by vortexing and then centrifuge at 10000 xg for 5 minutes. Recover the supernatant and transfer to a new tube. Store unused final sample at 4 °C. *Note: If activated charcoal is not available, then omit this step and pass the hydrolyzed sample through a 0.45 µm PVDF syringe filter unit. |
| Assay Procedure: | 1. Prepare and mix all reagents thoroughly before use. Each sample, unknown and standard should be assayed in duplicate.<br>2. Add 10 µL of unknown acid hydrolyzed samples to a 96 well microplate. Note: If needed, unknown samples may be diluted in water. If an oven is not available for incubating microplates at 60 °C, use microcentrifuge tubes for the assay and incubate in a waterbath. Transfer 150 µL of the final colorimetric reaction (after step 8 below) to a microplate for final absorbance reading.<br>3. Evaporate unknown acid-hydrolyzed samples under vacuum to dryness at 60-80 °C for 30-45 minutes. If a vacuum source is not available, evaporation may be performed on a heat block or in an oven. Note: Unknown samples must be dried to remove any residual HCl that could inhibit the colorimetric assay reaction. 5<br>4. Add 10 µL of each hydroxyproline standard to separate wells or tubes.<br>5. Add 100 µL of the Chloramine T Mixture to each well or tube.<br>6. Incubate for 30 minutes at room temperature.<br>7. Add 100 µL of Ehrlich's Reagent to each well or tube.<br>8. Incubate 90 minutes at 60 °C.<br>9. Read absorbance of each well on a microplate reader using 540-560 nm as the primary wave length. |
| Restrictions: | For Research Use only |

## Handling

| | |
|---|---|
| Storage: | 4 °C |
| Storage Comment: | Upon receipt, store the entire kit at 4°C. |

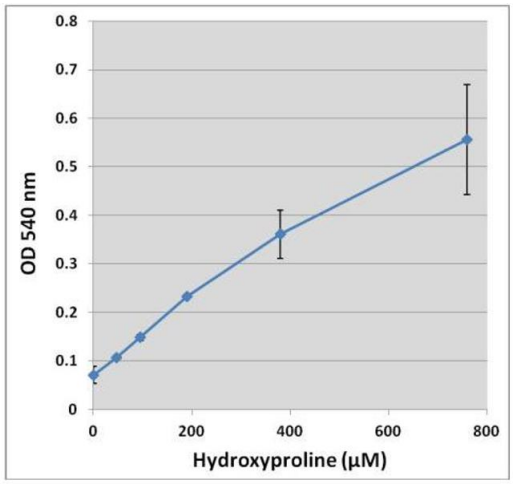
## Publications

| | |
|---|---|
| Product cited in: | Park, Lee, Park, Han, Sim, Park, Park: "Roflumilast Ameliorates Airway Hyperresponsiveness Caused by Diet-Induced Obesity in a Murine Model." in: **American journal of respiratory cell** |

**and molecular biology**, Vol. 55, Issue 1, pp. 82-91, (2016) (PubMed).

Liu, Pathak, Boehme, Chiang: "Cholesterol 7α-hydroxylase protects the liver from inflammation and fibrosis by maintaining cholesterol homeostasis." in: **Journal of lipid research**, Vol. 57, Issue 10, pp. 1831-1844, (2016) (PubMed).

## Images




**Biochemical Assay**

**Image 1.** Hydroxyproline standard curve.

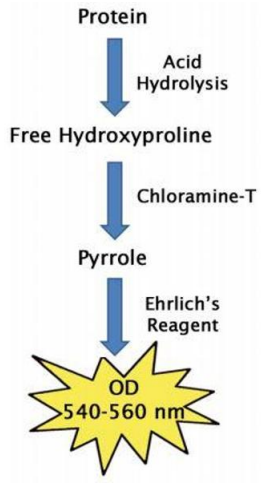
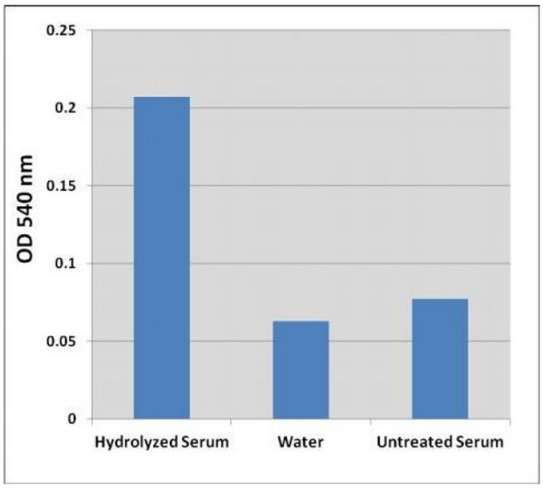



**Image 2.** Assay Principle




## Biochemical Assay

**Image 3.** Detection of hydroxyproline in human serum. Pooled human serum (or water as a negative control) was treated by acid hydrolysis according to the Preparation of Samples Section. Samples were tested according to the Assay Protocol.